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EFFECTS OF SUPERABUNDANT OXYGEN ON THERMAL TOLERANCE OF GOLDFISH

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The physiology and ecology of thermal tolerance in teleosts has long been the subject of research, and several extensive articles and reviews on this topic have appeared in recent years (Brett, 1956; Fry, 1958, 1967; Fisher, 1958; Weatherley, 1963a, b). Temperature tolerance is greatly affected by physiological processes, which are governed by the normally prevailing temperatures of the environment, and there has therefore been much study of the relationship between lethal temperature and acclimation temperature (*e.g.*, Brett, 1956; Fry, 1967). The fundamental biological characteristics of impairment of animal function through high temperature have, however, remained rather obscure (see Fry, 1967). Nor is there yet much understanding of the effects of environmental factors, other than acclimation temperature, on thermal tolerance. The importance of oxygen tension, salinity level, pH, *etc.*, are, for instance, largely unknown.

Hypothesis of Oxygen-Dependent Thermal Tolerance

Despite the lack of real understanding mentioned above, it has, at least, become evident that simplistic ideas of the overriding importance of some single cause of heat death are unlikely to be satisfactory (Rose, 1967). On the other hand, it does appear likely that certain aspects of the physiology of the whole animal will prove more directly sensitive to high temperature than others (Weatherley, 1963a). Thus, it has been thought for many years that functional failure of the nervous system may be of key importance (Brett, 1956; Fisher, 1958; Fry, 1958, 1967). This suggestion has considerable logical appeal in view of the long-known vulnerability of vertebrate nervous systems to even short-term deprivations of oxygen. We might, for instance, advance an argument along the following lines to account for impairment of the animal through damage to the nervous system when exposed to high temperature.

If ambient temperature rises substantially, then oxygen requirements of all tissues may be expected to show a corresponding increase. However, the circulatory system would be able to meet the initial need for an increase in blood flow, and so supply the additional oxygen required. Should their demand begin to exceed the maximum ability of the respiratory and circulatory systems to supply oxygen, some tissues may be expected to begin to suffer from an oxygen shortage. The severity and permanence of their resulting malfunction will then depend on their particular sensitivity to that shortage. Nervous tissue will be among those tissues—with a high inherent metabolic rate and marked sensitivity to oxygen lack—most rapidly and seriously affected. As the vertebrate respiratory/circulatory system is, at least partly, under the control of the nervous system, we can expect the estab-

lishment of a 'vicious cycle' of worsening failure of both systems, if the oxygen shortage is maintained. In addition, the systems for oxygen transport and for oxygen and carbon dioxide exchange in vertebrates may, of course, be directly affected by temperature.

An experimental finding for trout and roach by Alabaster and Welcomme (1962) lends support to these ideas. They found that if the oxygen tension in the water is significantly below air-saturation value, thermal tolerance is appreciably lowered.

The present paper describes experiments performed to test the hypothesis that the lethal temperature of goldfish may be affected by failure of the oxygen supply system. The method used was to augment the normal oxygen supply, in the expectation that a superabundance of oxygen might ensure ready entry of oxygen into the fish through the entire body surface. Thus, a functional failure of the respiratory, circulatory, or oxygen transport systems would be compensated, at least to some extent, by saturation of blood serum, lymph, tissue fluids, etc., with oxygen.

Materials and Methods

Plan of experiments

A first testing of the hypothesis was to compare mean survival time at a fixed upper lethal temperature among fish in oxygen-enriched water ('experimentals') with those in water at air-saturation ('controls'). The oxygen available to the 'controls' was limited to the quantity that dissolves in water at the altitude of Canberra (approx. 600 m). In this first group of experiments all goldfish were removed directly from an aquarium stock tank and placed immediately either into the oxygen-rich water or ordinary 'air-saturated' water in the experimental vessels.

In the second group of experiments batches of fish were placed in oxygen-rich water for periods of 2, $6\frac{1}{4}$ and $27\frac{1}{2}$ h before testing them against 'controls', or normal 'experimentals' (those placed in oxygen-rich water immediately before testing).

The results of these two groups of experiments (Tables I and II), indicated the desirability of determining the ability of very much higher concentrations of oxygen to ameliorate thermal stress. A third series of experiments was therefore carried out in a specially constructed compression chamber (Fig. 1), and the results are given in Figure 2.

The fish

The goldfish *Carassius auratus* used in these experiments, which were obtained from a commercial supplier, were mostly of the type known as 'comets', except for a few experiments (1 and 2, Table I) in which 'calicoes' were used. In any one series of experiments the fish were of relatively uniform size and were sexually immature. The fish were maintained in aerated, filtered water in glass aquarium tanks and received 12 hours of light and 12 of darkness per day. They received a standard fish food every two days, similar to that described by Weatherley (1963a), and for which they showed good appetite.

FIGURE 1. Compressor unit (4.4 l capacity) for experiments on upper lethal temperature at high tensions of oxygen. Chamber is constructed of stainless steel, with perspex end plate. Thermometer, pressure gauge, bleeder valve and high pressure oxygen hose are shown.

TABLE I

Survival times of goldfish in superoxygenated water compared with controls in air-saturated water when exposed to lethal temperature of 40°C. (approximate acclimation temperature 28° C).

Experiment No.	Treatment	No. of individuals n	Mean survival time (sec) $\bar{x}$	Sums of squares Σd^2	Standard deviation of diff. of means σd	't'	Significance level (%)
1	Control	8	192	64397	50.5	19.6	0.1
	High oxygen	8	1181	1245688			
2	Control	8	264	206988	67.3	10.8	0.1
	High oxygen	8	989	2117888			
3	Control	8	528	957552	80.1	8.0	0.1
	High oxygen	8	1170	2330400			
4	Control	6	587	323334	75.7	7.8	0.1
	High oxygen	6	1175	913350			
5	Control	8	203	152152	45.1	12.0	0.1
	High oxygen	8	746	890588			

In general, acclimation temperatures in the stock tanks varied by ± 0.5° C daily about the means as indicated below and in Tables I and II; Fig. 2. In any one series of experiments the fish were either drawn randomly from one large stock tank in which all had spent at least a fortnight prior to experiments, or from several

smaller adjacent tanks with identical temperature records. Sample bias is believed to have been kept to a minimum by these procedures.

Oxygen-supersaturated water at normal atmospheric pressure.

For the first series of experiments the test containers consisted of two large beakers, each containing approximately 2 liters of aquarium water (Table I). In the second series of experiments (Table II) the somewhat larger size of the fish required the use of even larger beakers of 5 liters capacity. In both series of tests a temperature was selected as 'lethal' on the basis of simple preliminary pilot tests combined with consideration of the published records of lethal temperatures

TABLE II

Analysis of survival of goldfish held in oxygen-saturated water for various times before exposure to a lethal temperature of 35° C, compared with controls in air-saturated water (approximate acclimation temperature 18° C).

Experiment No.	Treatment	No. of individuals	Range of survival times (sec)	Sums of E scores preceding each C score (Mann-Whitney 'U' test)	Significance level (%)
6	Control	8	70–555	—	—
7	Placed in O_2-sat. water immediately before test	8	195–>7200	4	0.1
8	2 hr in O_2-sat. water before test	8	160–>7200	14	3.2
9	$6\frac{1}{4}$ hr in O_2-sat. water before test	8	295–>7200	3	0.1
10	$27\frac{1}{2}$ hr in O_2-sat. water before test	8	180–>7200	12	1.9

C = 'control' values for survival times; E = 'experimental' values for survival times.

for goldfish at various acclimation temperatures (Brett, 1956). With these guides as a basis, 40° C proved convenient in experiments 1–5 (Table I), whereas in experiments 6–10 (Table II), because of differences in the size and age of the fish and their acclimation temperature, 35° C was used. Temperature was maintained in each beaker at the selected lethal value by placing them in a glass tank in which the water was kept at a constant temperature by a Braun 'Thermomix'. Temperatures in the beakers varied from the selected lethal temperature by no more than ± 0.05° C; in most instances no changes at all were detected.

In the 'control' beakers the water was aerated, with air at 3 lb per sq inch pressure passing through an aquarium airstone, for at least 10 min before each experiment. In the 'experimental' beakers water was supersaturated with oxygen by vigorous passage of pure oxygen for about 10 minutes.

Determinations of oxygen concentration in both containers at the atmospheric pressure of Canberra and the acclimation tempertures of these experiments (17–28° C) showed the saturation value to be 92–93% of the sea level value, and 4–5 times this as a result of superoxygenation. The oxygen tension in the 'experimental' beakers was thus always beyond the range of an oxygen electrode, and was therefore determined by the Winkler method, using 100 ml samples.

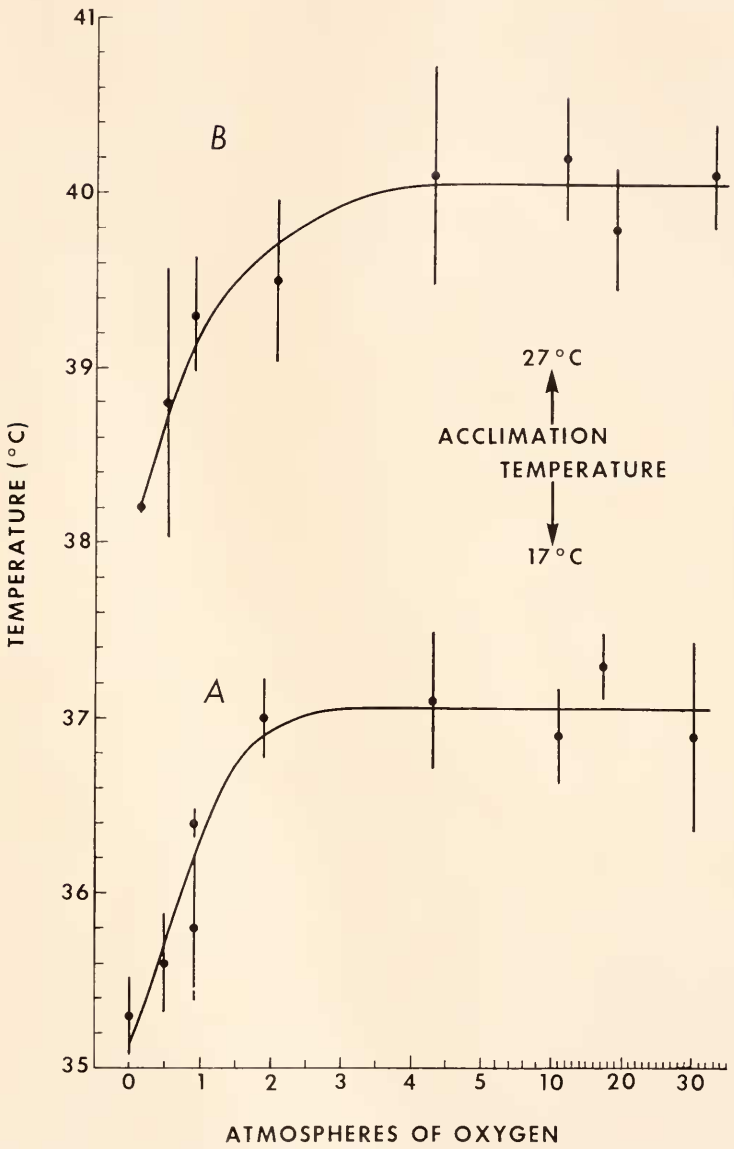

FIGURE 2. Effects of increasing concentrations of dissolved oxygen on mean upper lethal temperature (black circles), based on samples of five fish per point. Lower curve (curve A in text) was for fish acclimated at 17° C, upper curve (B in text) for fish acclimated at 27° C. Vertical bars represent twice the standard deviation of each mean. See text for further explanation.

In the beakers, the water was continuously aerated ('controls') or oxygenated ('experimentals') during the actual exposures of fish to lethal temperatures.

The method of testing was to expose samples, usually of 8, occasionally of 6 or 7, fish to the test temperatures by placing all fish simultaneously into the

beakers. Times to death were recorded for each individual. The criterion of death was cessation of movement, which was sometimes rather difficult to observe, though judgment rapidly improved during a few pilot tests. Cessation of opercular movement appeared to be closely correlated with that of other movements of the body, so it was frequently employed as a convenient and sensitive index of death. Many fish judged 'dead' by the criterion of 'cessation of movement' could be readily revived if removed immediately to a cold surface or cool water, but, by the same token, fish in which movement had ceased failed to move again if maintained at the same temperature or if the temperature was raised still higher.

On removal from the lethal temperature test beakers fish were weighed and measured. pH in the beakers was measured before and after each lethal test.

Higher concentrations of oxygen

The results of exposing goldfish to concentrations of oxygen higher than those obtainable by vigorous aeration alone (Tables I and II) made it desirable to examine the effects of much higher tensions of oxygen. The construction of a compression chamber was needed for this investigation (Fig. 1). The chamber was built of $\frac{1}{8}$ inch seamwelded stainless steel, in the form of a cylinder. A 1-inch-thick perspex end wall may be bolted to a heavy steel collar welded to one end; an airtight seal is effected by means of a rubber O ring. The unit carries a pressure gauge, a tap from which rubber pressure hose leads to an oxygen cylinder, and a steel-sheathed thermometer which dips into the water of the chamber when in use.

Before use, the compressor was placed on end and filled with water to a volume of 3.1 l (total capacity 4.4 l). Then oxygen was bubbled through this for 10 min to allow as much as possible to enter solution under normal atmospheric pressure. A randomly drawn sample of 5 goldfish was then placed in the water and the perspex end plate bolted on. With the chamber now set on its side, the air above the water was flushed out, with a powerful stream of oxygen, for one minute. With the outlet valve closed the pressure of the oxygen atmosphere above the water was then raised to whatever level was required.

Henry's Law states that the amount of gas which dissolves in a liquid is directly proportional to the partial pressure of the gas in the atmosphere above the liquid. The law was assumed to hold under the above experimental conditions, so that the tension of dissolved oxygen was computed from the recorded atmospheric pressure in the chamber (Fig. 2).

Apart from the higher oxygen concentrations obtained with this compression chamber it was also used to examine the influence on lethal temperature of various other concentrations of oxygen from a few per cent up to 400–500% of air saturation (Fig. 2). As in the first two series of experiments (Tables I and II) oxygen tensions of about 2 and about 4–5 atmospheres were obtained, without compression, merely by passing pure oxygen through the water. Tensions of oxygen less than air saturation were easily got by bubbling pure nitrogen through the water.

The aim in covering this very wide range of oxygen tensions (from near zero to about 30 atmospheres) was to test the influence of oxygen tension on thermal tolerance comprehensively. All oxygen tensions from 10–200% of air

saturation were determined by means of a temperature-compensated oxygen electrode.

Lethal temperatures were determined by placing the chamber containing each batch of fish, immediately after the end plate was bolted on (and after compression if needed), into a previously prepared glass tank. For experimental series A (Fig. 2) the water in this tank was held at 41.5° C, and for series B (Fig. 2) at 43.1° C. These temperatures had been carefully predetermined as a result of pilot tests to discover the temperatures at which fish, acclimated at 17 and 27° C, would die. These tank temperatures were known to be sufficient to heat the water of the compression chamber past the lethal level, but to do it slowly enough for the accurate estimation of the temperature at death (movement cessation) for each fish. The fish could be easily seen through the perspex wall of the compression chamber. The chamber was not constantly lighted, but a lamp was positioned so that it could be used to examine the rear end of the chamber if fish drifted away from the perspex.

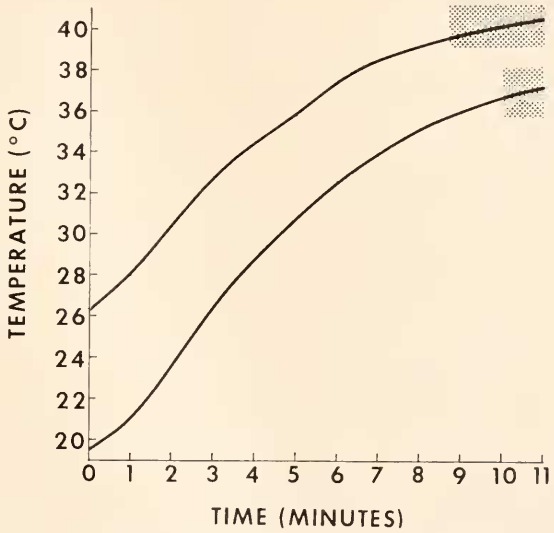

FIGURE 3. Rate of increase in temperature in compressor (Fig. 1) with external water temperature of 43.1° C (upper curve) and 41.5° C (lower curve). Hatching indicates range of temperature over which fish died in the experiment represented by each curve.

Figure 3 gives two curves depicting temperature rise against time in the chamber. The curves start at different values, each adjusted to the acclimation temperature of the animals. The curves pass through the actual temperatures recorded at one minute intervals: there has been no artificial smoothing.

It is noteworthy that rate of heating is not particularly significant in itself in influencing the temperature at which fish die. In several tests not reported here, the rate of heating to the lethal temperature was considerably more rapid than usual, though other conditions (i.e. oxygen tensions) were comparable. In these tests, mean lethal temperatures showed little difference as a result of the more rapid heating.

Results

Oxygen-saturated water at normal atmospheric pressure

In experiments 1–5 plus several not reported here, the mean survival times of goldfish exposed to a lethal temperature in oxygen-saturated water, as compared to those in aerated water, were about 2–5 times greater (Table I). The difference between these means was significant at the 0.1% level ('t' test; Fisher, 1954).

While they remained alive, the gills of the fish exposed in oxygen-rich water were also visibly redder than those in ordinary water. Suggestive evidence only, this observation nevertheless accords with the idea that respiratory/circulatory failure accompanies progressive physiological impairment in the thermally stressed fish, *i.e.* that blood flow through the gills becomes reduced more rapidly in response to a relative oxygen shortage.

The results of experiments 6–10 (Table II), dealing with fish held at high oxygen tensions for various periods before exposure to the lethal temperature again showed a strong tendency for survival to be much longer than in ordinary aerated water. However, though the 'control' goldfish all died within a few minutes, the fish in oxygen-rich water showed a pronounced tendency to survive so much longer in all 'experimental' instances that each test was terminated with some fish still alive after 2 h. In testing the significance of the difference in survival times between 'experimental' and 'control' groups the Mann-Whitney non-parametric 'U' test was used instead of the 't' test (Siegel, 1956).

Results show that survivals in oxygen-rich water were all significantly better than in air-saturated water, but that fish placed directly in a high oxygen tension at the beginning of their 'lethal' test survived at least as well as those given longer periods in high oxygen tensions before testing. This disposes of the possibility that giving fish prolonged exposures to high oxygen *before* exposure to lethal temperature tests might cause them to survive even longer than those placed directly in high oxygen only at the beginning of the test.

In experiments 1–5 of Table I the pH in the control beakers was 7.5, and of the oxygen-rich ones 7.9; pH measured immediately before and after lethal tests showed no changes. Weights ranged from 0.4–1.3 g (mean 0.7 g) for 'calicoes' in experiments 1 and 2; from 0.3–1.1 (mean 0.7 g) for 'comets' in experiments 3 and 4; for experiment 5 the mean weight was 0.2 g.

In experiments 6–10 (Table II) pH of controls was 7.4 and in the oxygenated water was 7.6, whether after 2, $6\frac{1}{4}$ or $27\frac{1}{2}$ hr passage of oxygen. Weights of fish in this series ranged from 2.2–3.9 g (mean 3.0 g).

Higher concentrations of oxygen

Upper lethal temperature was obviously related closely to oxygen tension over a considerable range of values (Fig. 2). Mean lethal temperature increased in both series of experiments (*i.e.* with fish acclimated to 17 and 27° C, respectively) by about 2° C, from a low corresponding to oxygen tensions of about 10% of air saturation up to a maximum at approximately 5 atmospheres. This tension of oxygen is also about the same as that which produced highly significant increases in survival time in the first two series of experiments. Thus, increased ability to survive a high temperature because of oxygen abundance can be manifested either by

increased survival time at a lethal temperature or by a higher mean lethal temperature achieved. There is nothing remarkable about these parallel associations; Fry (1967) has pointed out that they are only to be expected. It is, however, reassuring to find them demonstrated within one experimental study.

There was no additional enhancement of thermal tolerance in response to exposure to oxygen tensions in excess of 5 atmospheres—even up to approximately 30 atmospheres. The limit was reached quite abruptly. On the other hand, there was no evidence of oxygen toxicity effects at higher tensions.

In these experiments pH varied only over the range 7.4–7.5 in the compression chamber at the beginning of the tests. Weights in series A ranged from 2.9–4.4 g and in series B from 3.0–4.8 g.

Discussion

The results do not appear to be out of harmony with the hypothesis on which the experiments were based, so perhaps it may be seriously contended that some important malfunction of mechanisms that supply oxygen to the tissues begins at or near the lethal temperature level. Two arguments may be advanced in support of this. The first is that temperature tolerance is lowered when oxygen tensions are appreciably reduced below normal air saturation values; the second is that goldfish can survive for long periods in concentrations as low as 8% of air saturation at a temperature of 20° C (own unpublished observations). Blažka (1958) has shown that the closely related crucian carp *C. carassius* can live under conditions of almost if not complete anoxia for several months at low temperatures. So it seems that oxygen insufficiency is a relative problem for fish, exacerbated particularly when temperatures are dangerously high. This reasoning accords with the hypothesis of damage to respiratory, circulatory, and oxygen transport, systems. The fact that there is an increase in thermal tolerance in response to increased oxygen, of an order similar to the decrease in tolerance seen in oxygen-poor conditions, also supports the hypothesis—or certainly does it no violence.

On the other hand the suddenly achieved limit of this enhancement of tolerance suggests the operation of a new effect at an apparently very definite 'breakpoint', and here we may be witnessing a physiological failure of a quite critical sort. The enhancement of thermal tolerance up to 5 atmospheres of oxygen ends so abruptly that one must suppose not that more oxygen cannot be accepted by the tissues and body fluids of the fish, but simply that it is unable to utilize this extra oxygen. There might be several reasons for this and in concluding I list these.

(i) Heat denaturation of proteins including respiratory enzyme systems may make it impossible for the fish, as a whole organism, to make full use of the oxygen available to it.

(ii) Heat destruction of the lipoprotein of the cell membrane may make oxygen import into the cell, and/or carbon dioxide export out of it, impossible. At one time lipid was certaintly envisaged as important in thermal tolerance of goldfish (Hoar and Cottle, 1952a, b; Hoar and Dorchester, 1949).

(iii) Direct thermal death or irreversible damage of cells of the central nervous system may be quite unpreventable above a certain temperature maximum despite the presence of a superabundance of oxygen.

Any or all of the above effects may be at work; but perhaps some *system* critically linked to temperature may be responsible. If the latter proved correct it would be very interesting in several ways. One of these is a major point of this paper, which is to suggest that another system (or systems)—apparently failing at a critical level of temperature or after a critical exposure time—can have its failure alleviated merely by experimental amelioration of some accompanying environmental conditions. In that case it might be possible to postulate even more significant alleviations (if we could determine other ameliorating conditions for the new limiting factors).

Summary

A hypothesis may be made out that the respiratory/circulatory, and possibly the oxygen transport, systems are importantly involved in thermal death of goldfish. Experiments showed that at two distinct levels of temperature acclimation, superabundant oxygen in the water could produce a definite improvement either in time of survival at a fixed lethal temperature or in lethal temperature reached as a result of heating.

Despite this positive effect of high oxygen a clear cut failure of oxygen, in excess of about 5 atmospheres (partial pressure), to produce further improvement in thermal tolerance suggests the failure of some critical system directly affected by temperature.

The experiments, while revealing nothing of the detailed mechanisms involved, certainly do not invalidate the hypothesis proposed, and open a way to further investigation.

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